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A Cytotaxonomic Study of Some Ferns from Jiangsu and Zhejiang Provinces, China

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The chromosome numbers and putative reproductive manners (in most) of 31 species and 2 varieties of ferns from Jiangsu and Zhejiang, central eastern China, were examined. Data include a few new records as regards species, variety or cytotypes. *Pteris dispar* has diploids in a restricted distribution range and tetraploids in a wide range in East Asia. *Microlepia marginata* has the basic number x=42 (not 43), and var. *bipinnata* is a triploid hybrid. *Cyrtomium fortunei* includes sexual diploids and apogamous triploids. *Dryopteris pacifica* has apogamous diploids and triploids as do Japanese populations, and *D. chinensis* includes apogamous tetraploids. The basic number of *Parathelypteris glanduligera* is x=40.

Cytological and reproductive variations play significant roles for fern evolution, diversity, and biogeography (Manton 1950; Lovis 1977; Walker 1979, 1984; Wagner and Wagner 1980; Kato et al. 1992). There exists a high correlation between polyploid, particularly triploid, and apogamous reproduction (Wagner and Wagner 1980). About 10% of ferns in the world are apogamous (agamosporous, apomictic), but the frequencies of apogamous species in local floras are various (Lovis 1977; Walker 1979). For example, the frequency (17%) in Japan is relatively high (Mitui 1975, 1980; Masuyama 1984), indicating a great contribution of apogamous species to the diversity of the Japanese fern flora.

Continental China is the largest and central region in East Asia and surrounded by neighboring smaller regions. While southwestern China is floristically close to the Himalayas, the eastern part is closely related to Japan, Korea, and Taiwan (Ching 1976). Particularly, the fern flora of the coastal and near-coastal central China including Jiangsu Province includes very many species that are identical to Japanese species. For example, 98 (75%) of totally 130 species in Jiangsu Province including a few cultivated species (Botanical Institute of Jiangsu Province 1977) are also distributed in Japan. Therefore, in order to understand further the floristic and biogeographic relationships of East Asian ferns, detailed comparative studies of the floras of eastern China and Japan covering cytology and reproduction are waited.

A number of cytotaxonomical studies have been made for Chinese ferns (Roy and Holttum 1965; Chiu 1981; Wang 1982, 1985, 1987, 1989a, b; Wang and Zhang 1981; Wang and Sun 1982; Wang and Xia 1984; Wang et al. 1984; Weng 1985a, b, 1989a, b, 1990; Weng and Qiu 1988; Li 1989; Kato et al. 1992). So far, they record about 270 species, which account for 10–15% of all Chinese fern species. Among others, Kato et al. (1992) reported the chromosome numbers and reproductive manners of 90 species from Yunnan, southwestern China. However, much more data are needed to examine the cytotaxonomy and phytogeography of eastern Chinese ferns.

Kato and his colleagues carried out field studies in Jiangsu and Zhejiang Provinces, central eastern China, in 1991, and Nakato and Kato, in Jiangsu in 1992. In this region with a close phytogeographic affinity to Japan as reviewed above, we collected materials for the present cytological study in an attempt to investigate cytotaxonomical and cytogeographical relationships of eastern Chinese ferns.

Materials and Methods

The materials examined in this study and their collecting localities are shown in Table 1. Voucher specimens are deposited in the herbarium, Botanical Gardens, Faculty of Science, University of Tokyo (TI). For observing somatic chromosome numbers, root tip cells were pretreated in 0.002 M 8-hydroxyquinoline solution for 3 to 6 hours. They were fixed in 45% acetic acid for 20 minutes, hydrolyzed in a mixture of 1N HCl and 45% acetic acid (3:1) at 60°C for 1 minute, stained with aceto-orcein, and squashed. For indirectly determining reproductive manners, the number of spores produced in single sporangia was counted for the plants cytologically examined. Thirty-two-spored plants were inferred to be apogamous, while 64-spored ones, sexual (Walker 1979).

Results and Discussion

The results of this study are shown in Table 1 and Figs. 2–31. Thirty-one species and two varieties belonging to 15 genera of nine families were determined. They include new chromosome numbers and reproductive manners of some species or cytotypes, which are marked in Table 1, and cytotaxonomically and biogeographically noteworthy species are described and discussed below.

Pteris dispar — Nakato (1981) described that diploids of this species are distributed in the Ryukyu Islands and Taiwan, a region which is south of the isothermoline of annual mean temperature 20°C, while tetraploids occur in Kyushu, Shikoku, and Honshu that are located in a zone north of this line and south of the isothermoline of annual mean temperature 14°C. Nakato also suggested from spore size that plants from Guangdong, southern China, are tetraploid. Diploids and tetraploids are recorded from Zhejiang, central eastern China (Chiu 1981; Weng and Qiu 1988) and tetraploids from Jiangsu north of Zhejiang (present study). The available data are basically consistent with Nakato's (1981) hypothesis, showing the restricted distribution range of diploids in the Ryukyu islands, Taiwan, and eastern China, in contrast to the wide range of tetraploids and assumed tetraploids in southern and eastern China, Japan, and southern Korea (Figs. 1, 25; Table 1).

Microlepia marginata — All plants examined from Japan including the Ryukyu Islands except 2 plants from Jinmuji (Kanagawa Pref.), Taiwan, and China are tetraploids (Mitui 1968; Tsai 1972; Nakato and Serizawa 1981; Tsai and Shieh 1983; Weng 1985a; Weng and Qiu 1988; Yang et al. 1988; Nakato, unpubl. data; present study, Fig. 4). The Jinmuji plants are hexaploid, and diploids are not reported.

Var. *bipinnata* from Japan is triploid (Mitui 1968; Nakato 1990) and tetraploid (Nakato and Serizawa 1981). Nakato and Serizawa (1981) and Nakato (1990) inferred from abnormal sporogenesis that the triploid var. *bipinnata* may be a hybrid between *M. marginata* (4x) and *M. strigosa* (2x), and the tetraploid var. *bipinnata* between *M. marginata* (6x) and *M. strigosa* (2x) or between *M. marginata* (4x) and *M. sinostrigosa* (4x). The triploid var. *bipinnata* examined from Jiangsu (Fig. 5) has many abortive spores as well as several more or less normal ones in each sporangium, suggesting a hybrid origin.

All previous studies on Chinese ferns (Tsai 1972;

Table 1. Species and families, somatic chromosome numbers, the numbers of spores produced in each sporangium, and localities and vouchers for ferns from Jiangsu and Zhejiang Provinces

Family/Species	Somatic chromosome no. (Ploidy level)	Spore no.*	Locality & voucher**
Blechnaceae			
Woodwardia japonica (L. f.) Sm.	c. 68 (2x)	64	Jiangsu. Yixing: Laoyingjai near Mt. Longchi; KN45
	68 (2x)	>46	Zhejiang. Hangzhou: Lingyin temple; KIS64 (Fig. 2)
Davalliaceae			
Davallia mariesii Moore	80 (2x)	64	Jiangsu. Liangyungang: Liuhe Nat. Res.; KN92 (Fig. 3)
Dennstaedtiaceae			
Microlepia marginata (Houtt.) C. Chr.	168 (4x)	38–50	Jiangsu. Liyang: Hengjian; KN85 (Fig. 4)
M. marginata var. *bipinnata* Makino	c. 126 (3x)	mostly abortive	Jiangsu. Yixing: Mt. Longchi; KN37 (Fig. 5)
Dryopteridaceae			
Arachniodes liyangensis Ching et Y.C. Lan (?)***	82 (2x)	–	Jiangsu. Liyang: Hengjian; KN80 (Fig. 6)
A. pseudoaristata (Tagawa) Ohwi	c. 80 (2x)	–	Jiangsu. Yixing: Laoyingjai near Mt. Longchi; KN54
A. simplicior (Makino) Ohwi	82 (2x)	64	Jiangsu. Liyang: Hengjian; KN84 (Fig. 7)
Cyrtomium fortunei J. Sm.	82 (2x)***	64	Jiangsu. Suzhou: Guangfu Nat. Res., Wu Co.; KIS9 (Fig. 8)
	123 (3x)	32	Jiangsu. Juron. Co.: Mt. Baohua; KN1
	123 (3x)	32	Jiangsu. Yixing: Mt. Longchi; KN33 (Fig. 9)
	c. 123 (3x)	32	Jiangsu. Yixing: Laoyingjai near Mt. Longchi; KN48
	c. 123 (3x)	32	Jiangsu. Nanjing: Nanjing Bot. Gard.; KN122
Dryopteris atrata (Wall.) Ching	82 (2x)	54–60	Jiangsu. Liyang: Hengjian; KN75
	82 (2x)	64	Zhejiang. Hangzhou: Yunqi Bamboo Path; KIS51 (Fig. 10)
D. bissetiana (Bak.) C. Chr.	c. 123 (3x)	32	Jiangsu. Liyang: Hengjian; KN83
	c. 123 (3x)	32	Jiangsu. Liangyungang; Liuhe Nat. Res.; KN98
	c. 123 (3x)	32	Jiangsu. Liangyungang; Sucheng Nat. Res.; KN100
	c. 123 (3x)	ca. 30	Jiangsu. Liangyungang; Sucheng Nat. Res.; KN103
	123 (3x)	32	Jiangsu. Liangyungang; Mt. Huagou; KN112 (Fig. 11)
D. championii (Benth.) C. Chr. ex Ching	c. 123 (3x)	32	Jiangsu. Juron Co.: Mt. Baohua; KN4
	123 (3x)	32	Jiangsu. Juron Co.: Mt. Baohua; KN8 (Fig. 12)
	c. 120 (3x)	32	Jiangsu. Yixing: Mt. Longchi; KN26
	c. 123 (3x)	–	Jiangsu. Nanjing: Nat. Res. in Nanjing Bot. Gard.; KN119
	123 (3x)	32	Jiangsu. Suzhou: Guangfu Nat. Res., Wu Co.; KIS18

D. chinensis (Bak.) Koidz.	123 (3x)	32	Jiangsu. Liangyungang; Mt. Huagou; KN115 (Fig. 13)
	164 (4x)***	32	Jiangsu. Juron Co.: Mt. Baohua; KN7
	c. 164 (4x)	32	Jiangsu. Juron Co.: Mt. Baohua; KN12
	164 (4x)	32	Jiangsu. Juron Co.: Mt. Baohua; KN13 (Fig. 14)
	c. 160 (4x)	32	Jiangsu. Yixing: Mt. Longchi; KN23
D. erythrosora (Eat.) C. Chr.	123 (3x)	32	Jiangsu. Yixing: Mt. Longchi; KN30 (Fig. 15)
	123 (3x)	32	Jiangsu. Yixing: Laoyingjai near Mt. Longchi; KN57
	c. 123 (3x)	32	Jiangsu. Nanjing: Nat. Res. in Nanjing Bot. Gard.; KN131
D. fuscipes C. Chr.	123 (3x)	–	Jiangsu. Yixing: Mt. Longchi; KN21 (Fig.16)
	c. 123 (3x)	32	Jiangsu. Nanjing: Nat. Res. in Nanjing Bot. Gard.; KIS32
D. fuscipes var. *diplazioides* (Christ) Ching	c. 123 (3x)***	32	Jiangsu. Yixing: Mt. Longchi; KN31
	123 (3x)	32	Zhejiang. Hangzhou; Lingyin temple; KIS60 (Fig. 17)
D. gymnophylla (Bak.) C. Chr.	82 (2x)	64	Jiangsu. Juron Co.: Mt. Baohua; KN9
	82 (2x)	64	Jiangsu. Juron Co.: Mt. Baohua; KN10 (Fig. 18)
	82 (2x)	64	Jiangsu. Liyang: Hengjian; KN78
D. liyangensis Ching et Y.C. Lan(?)***	c. 120 (3x)	32	Jiangsu. Yixing: Laoyingjai near Mt. Longchi; KN65 (Fig. 19)
D. pacifica (Nakai) Tagawa	82 (2x)	32	Jiangsu. Yixing: Laoyingja near Mt. Longchi; KN60 (Fig. 20)
	123 (3x)	32	Jiangsu. Juron Co: Mt. Baohua; KN6
	c. 123 (3x)	32	Jiangsu. Yixing: Mt. Longchi; KN24
	c. 123 (3x)	32	Jiangsu. Yixing: Mt. Longchi; KN34
	123 (3x)	32	Jiangsu. Yixing: Mt. Longchi; KN36
	c. 123 (3x)	32	Jiangsu. Liangyungang; Liuhe Nat. Res.; KN87
	123 (3x)	32	Jiangsu. Liangyungang; Liuhe Nat. Res.; KN91 (Fig. 21)
	123 (3x)	32	Jiangsu. Liangyungang; Mt. Huagou; KN111
	123 (3x)	32	Jiangsu. Nanjing: Nat. Res. in Nanjing Bot. Gard.; KIS41
D. sacrosancta Koidz.	c. 123 (3x)	32	Jiangsu. Liangyungang; Liuhe Nat. Res.; KN95 (Fig. 22)
D. uniformis Makino	c. 164 (4x)	64	Jiangsu. Juron Co.: Mt. Baohua; KN16 (Fig. 23)
D. varia (L) O. Kuntze	c. 123 (3x)	32	Jiangsu. Nanjing: Nanjing Bot. Gard.; KN140
Polystichum tripteron (Kunze) Presl	c. 82 (2x)	–	Jiangsu: Liangyungang: Sucheng Nat. Res.; KN105
Polypodiaceae			
Pyrrosia lingua (Thunb.) Farewell	c. 70 (2x)	–	Jiangsu. Yixing: Laoyingjai near Mt. Longchi; KN67
P. petiolosa (Christ) Ching***	c. 72 (2x)	–	Jiangsu. Liangyungang: Liuhe Nat. Res.; KN90 (Fig. 24)

Pteridaceae			
Pteris dispar Kunze	116 (4x)	64	Jiangsu. Liyang: Hengjian; KN79 (Fig. 25)
Sinopteridaceae			
Onychium japonicum (Thunb.) Kunze	>106 (4x)	–	Jiangsu. Nanjing: Nat. Res. in Nanjing Bot. Gard.; KN133
Thelypteridaceae			
Phegopteris decursive-pinnata (van Hall.) Fée	120 (4x)	c. 60	Jiangsu. Liyang: Hengjian; KN73 (Fig. 26)
	120 (4x)	c. 60	Jiangsu. Liyang: Hengjian; KN81
	c. 120 (4x)	c. 60	Jiangsu. Nanjing: Nanjing Bot. Gard.; KN129
Parathelypteris glanduligera (Kunze) Ching	80 (2x)***	–	Jiangsu. Juron Co.: Mt. Baohua; KN18
	80 (2x)	64	Jiangsu. Liayang: Hengjian; KN82 (Fig. 27)
	c. 80 (2x)	54–58	Jiangsu. Liangyungang: Liuhe Nat. Res.; KN89
P. japonica (Bak.) Ching	c. 120 (4x)	64	Jiangsu. Liangyungang: Liuhe Nat. Res.; KN88
Woodsiaceae			
Athyrium niponicum (Mett) Hance	c. 80 (2x)	48–54	Jiangsu. Yixing: Laoyingjai near Mt. Longchi; KN68
	80 (2x)	64	Jiangsu. Liangyungang: Sucheng Nat. Res.; KN102 (Fig. 28)
Deparia okuboana (Makino) Kato	c. 120 (3x)	32	Jiangsu. Yixing: Laoyingjai near Mt. Longchi; KN58
	120 (3x)	32	Jiangsu. Liyang: Hengjian; KN74 (Fig. 29)
D. petersenii (Kunze) Kato	c. 160 (4x)	64	Jiangsu. Liyang: Hengjian; KN76 (Fig. 30)
Diplazium mesosorum (Makino) Koidz.	82 (2x)	64	Jiangsu. Juron Co.: Mt. Baohua; KN15 (Fig. 31)

*Specimens with prefix KN were collected by Kato and Nakato in 1992, and those with KIS, by Kato, Ito, and Saito in 1991.
**The number of spores produced in each sporangium.
***New record as to species, variety, or cytotype.

Tsai and Shieh 1983; Weng 1985a; Weng and Qiu 1988; Yang et al. 1988) described that the basic chromosome number of *Microlepia marginata* is 43, whereas Nakato and Serizawa (1981) reported x=42 for Japanese populations. Our observation showed clearly the same number as Japanese plants, x=42, in Jiangsu population.

Cyrtomium fortunei — In his palynological study Mitsuta (1986) inferred that 23 Chinese "species" (tentatively recognized by Mitsuta) are sexual (64-spored), 22 "species" are apogamous (32-spored), and 7 "species" include both sexual and apogamous plants. Of the last, Chinese *C. macrophyllum, C. yamamotoi* (=*C. fortunei* var. *intermedium*), and *C. omeiense* (which, according to Mitsuta, probably is conspecific with Japanese *C. macrophyllum* var. *tukusicola*) include sexual and apogamous plants, while all Japanese plants are apogamous. A similar case is *C. fortunei*. We found a sexual diploid plant for the first time (Table 1). Thus, Chinese *C. fortunei* contains sexual diploids, sexual tetraploids, and apogamous triploids (Weng and Qiu 1988; Weng 1990; Kato et al. 1992; present study, Figs. 8, 9), whereas only apogamous triploids are found in Japan

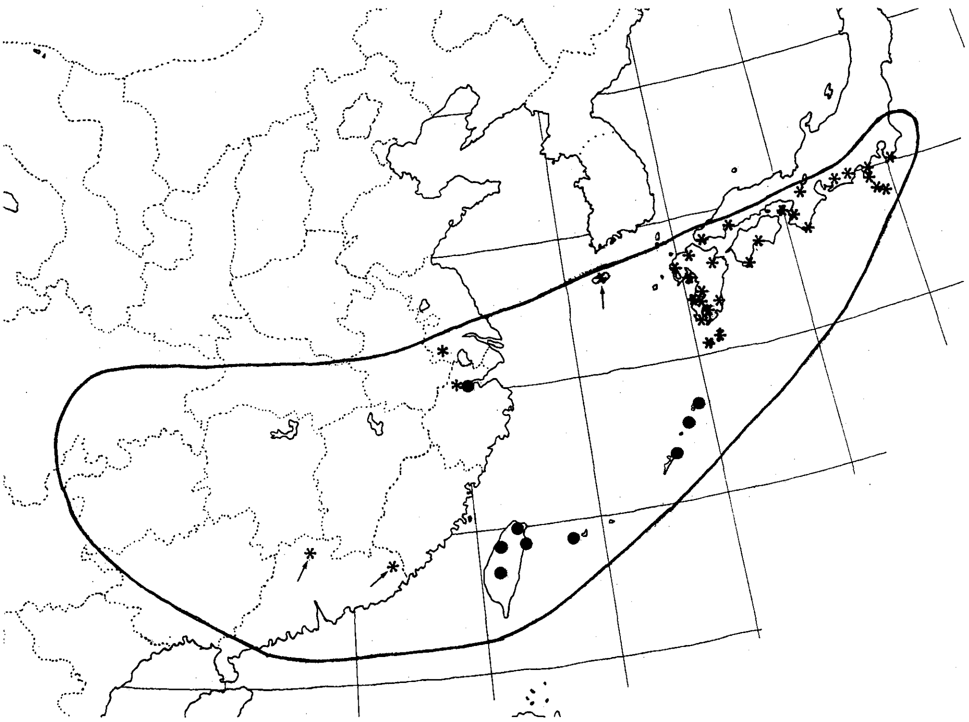


Fig. 1. Map showing the localities of two cytotypes of *Pteris dispar*. Solid circles, diploids (2n=58); asterisks, tetraploids (2n=116). Data are collected from Nakato (1981), Chiu (1981), Tsai and Shieh (1983), Weng and Qiu (1988). Arrows indicate putative tetraploids (Nakato 1981).

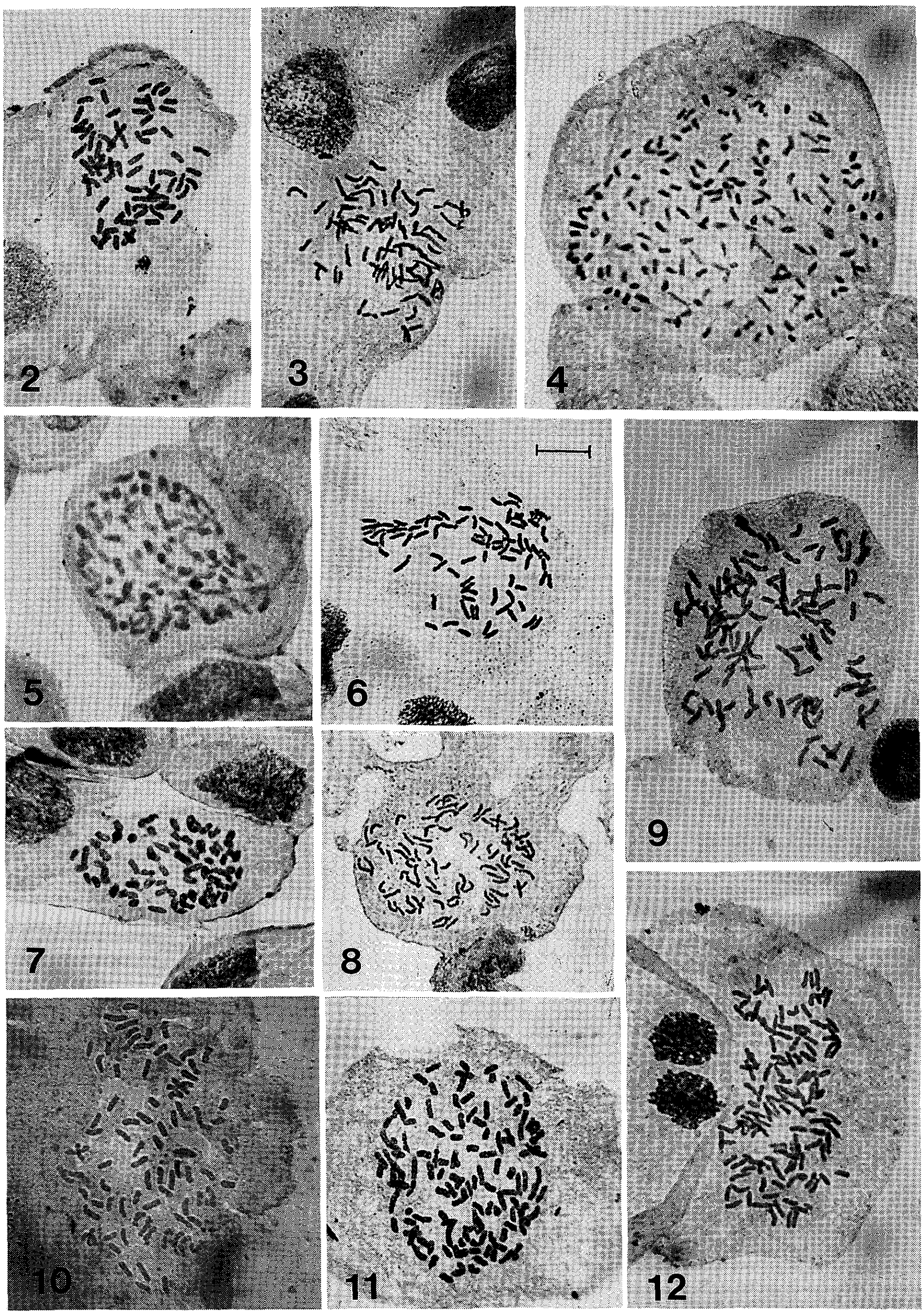
(Kurita 1960; Mitui 1968, 1980; Hirabayashi 1970; Matsumoto and Shimura 1985). Another case is *Asplenium* sect. *Hymenasplenium.* In a few identical species, both sexual and apogamous plants occur in Yunnan, southwestern China, while only apogamous plants are found in Japan (Murakami and Hatanaka 1988; Murakami, unpubl. data). On the other hand, *C. falcatum* in China and Japan includes sexual diploids, sexual tetraploids, and apogamous triploids (Matsumoto and Shimura 1985; Mitsuta 1986; Weng and Qiu 1988).

These examples indicate that Chinese fern flora is more diverse than the Japanese even at the infraspecific level (Kato et al. 1992). The distribution pattern of the cytological and reproductive variants of *Cyrtomium fornunei* suggests that the apogamous forms may have arisen from the sexual forms in China by autopolyploidization followed by apogamization and expanded to Japan.

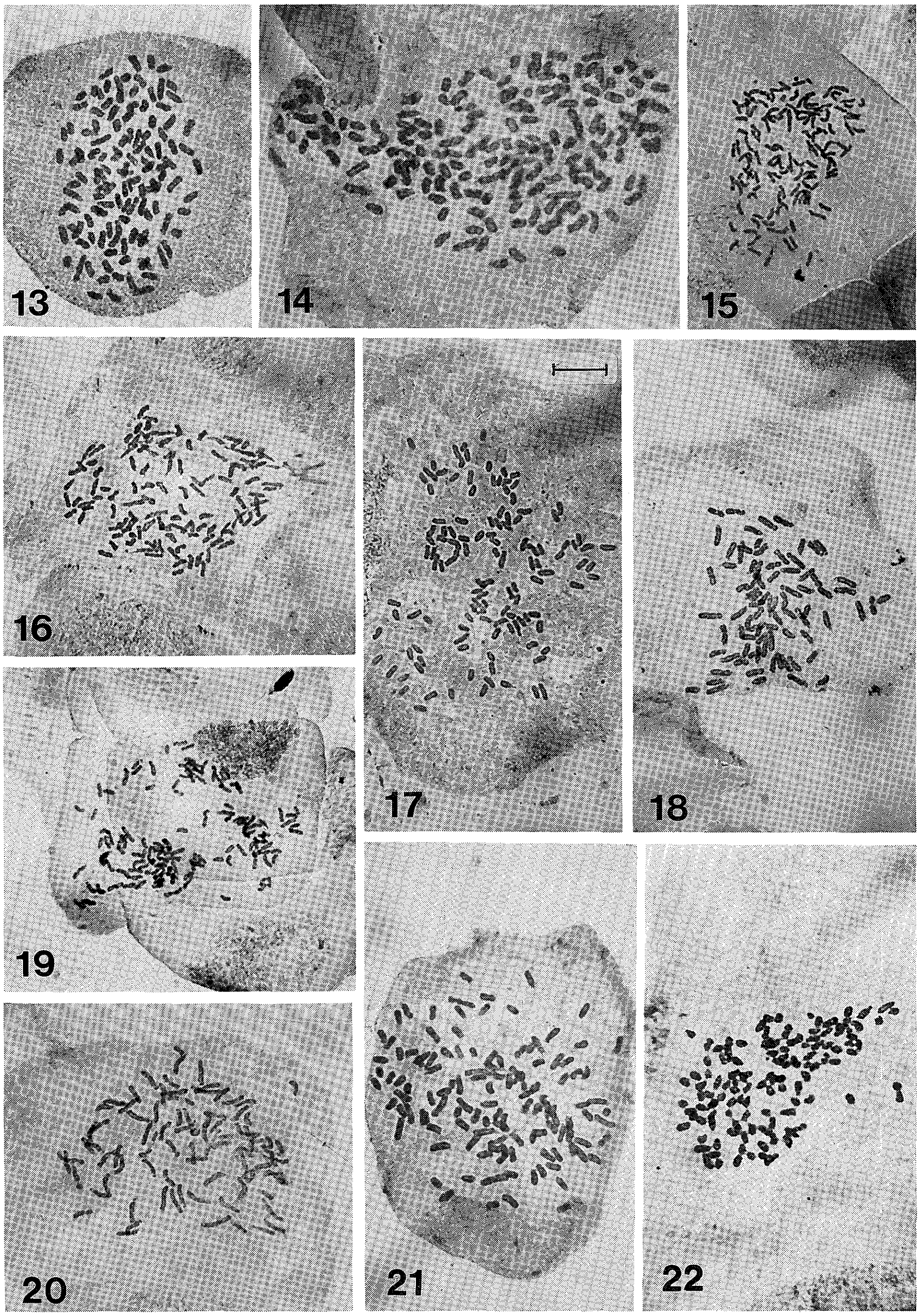
Dryopteris — This is a temperate genus which contains many identical or closely related species occurring in China and Japan. Of the 12 species and one variety examined here, *D. atrata* and *D. gymnophylla*, and *D. uniformis* are diploid and tetraploid, respectively, and reproduce sexually, while all the others are apogamous (Figs. 10, 18, 23; Table 1). Included in the latter is *D. liyangensis* , which was examined cytologically for the first time, although identification is tentative.

In all species examined, except the *D. chinensis* tetraploids, *D. fuscipes* var. *diplazioides*, and *D. liyangensis*, Chinese and Japanese plants show the same ploidy levels and reproductive manners, indicating a close biogeographic affinity.

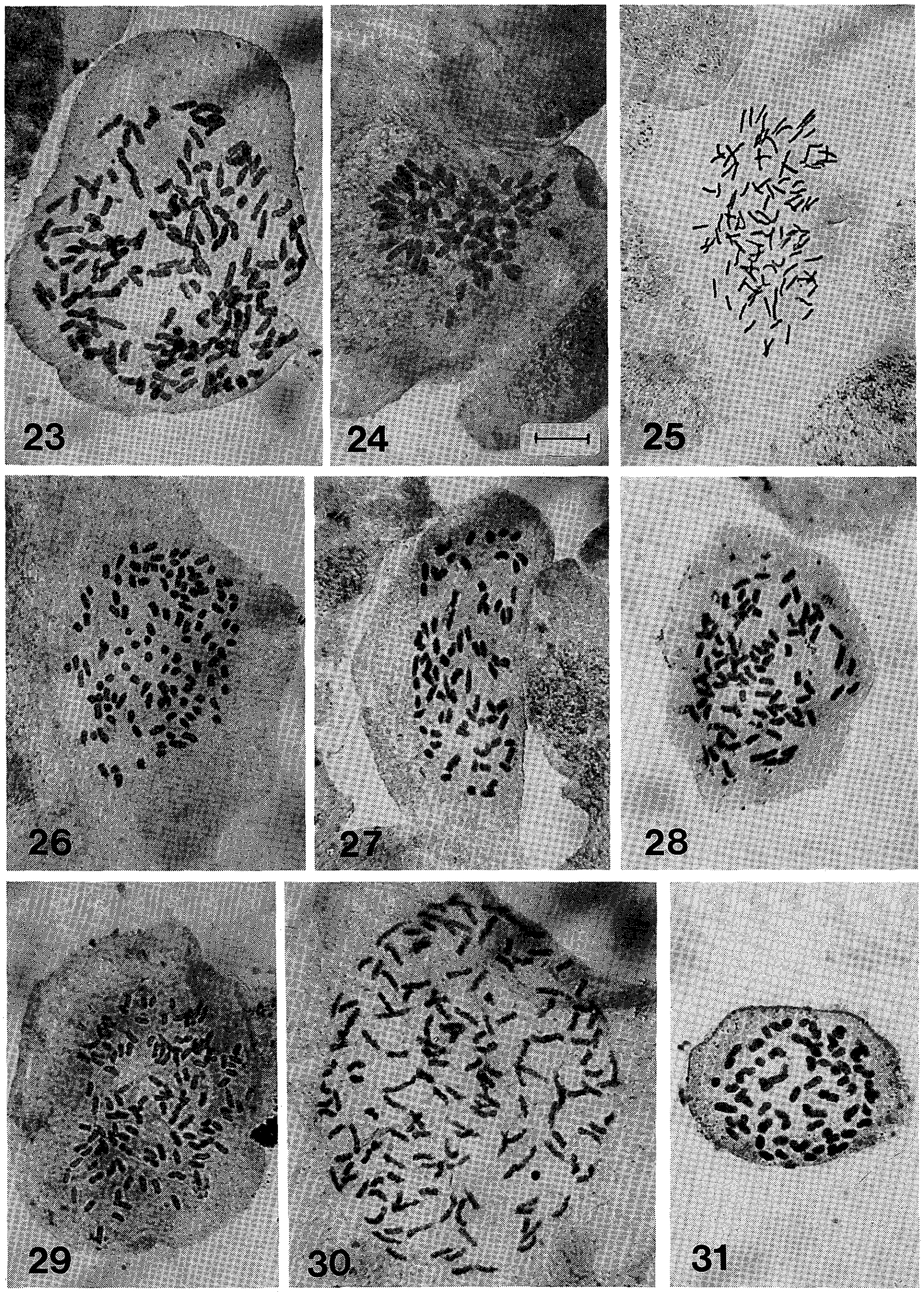
Dryopteris bissetiana, D. pacifica, D. sacrosancta, and *D. varia* of the *D. varia* group were determined to



Figs. 2–12. Photographs of somatic chromosomes. Scale bar = 10 μm for all figures. 2. *Woodwardia japonica*, 2n=68. 3. *Davallia mariesii*, 2n=80. 4. *Microlepia marginata*, 2n=168. 5. *Microlepia marginata* var. *bipinnata*, 2n=c. 126. 6. *Arachniodes liyangensis*(?), 2n=82. 7. *Arachniodes simplicior*, 2n=82. 8. *Cyrtomium fortunei*, 2n=82. 9. *Cyrtomium fortunei*, 2n=123. 10. *Dryopteris atrata*, 2n=82. 11. *Dryopteris bissetiana*, 2n=123. 12. *Dryopteris championii*, 2n=123.



Figs. 13–22. Photographs of somatic chromosomes. Scale bar = 10 μm for all figures. 13. *Dryopteris chinensis*, 2n=123. 14. *Dryopteris chinensis*, 2n=164. 15. *Dryopteris erythrosora*, 2n=123. 16. *Dryopteris fuscipes*, 2n=123. 17. *Dryopteris fuscipes* var. *diplazioides*, 2n=123. 18. *Dryopteris gymnophylla*, 2n=82. 19. *Dryopteris liyangensis*(?), 2n=c. 120. 20. *Dryopteris pacifica*, 2n=82. 21. *Dryopteris pacifica*, 2n=123. 22. *Dryopteris sacrosancta*, 2n=c. 123.



Figs. 23–31. Photographs of somatic chromosomes. Scale bar = 10 μm for all figures. 23. *Dryopteris uniformis*, 2n=c. 164. 24. *Pyrrosia petiolosa*, 2n=c. 72. 25. *Pteris dispar*, 2n=116. 26. *Phegopteris decursive-pinnata*, 2n=120. 27. *Parathelypteris glanduligera*, 2n=80. 28. *Athyrium niponicum*, 2n=80. 29. *Deparia okuboana*, 2n=120. 30. *Deparia petersenii*, 2n=c. 160. 31. *Diplazium mesosorum*, 2n=82.

be apogamous. *Dryopteris pacifica* included rare diploids and common triploids, while all other species are triploid (Figs. 20, 21; Table 1). As regards Chinese materials, it was reported that *D. bissetiana* is a sexual tetraploid, and *D. pacifica* and *D. varia* are apogamous triploid species (Weng 1989a, b), while Japanese plants of these species are apogamous diploids and triploids (Hirabayashi 1974; Mitui 1975). Although polyploidization is a predominant evolutionary trend, changes between low and high ploidy levels may be reversible in certain extreme cases such as *Dryopteris pacifica* (Lin et al. 1992). Lin et al. (1992) described that in *D. pacifica*, a triploid progenitor produces diploid as well as triploid progeny apogamously via particular sporogenesis accompanying ploidy reduction. Our finding indicates that apogamous diploids and triploids coexist in the Chinese as well as the Japanese *D. varia* group. On the other hand, if it is true that *D. bissetiana* includes sexual tetraploids, they might have been involved in hybrid speciation, which produces a high variation of the *D. varia* group.

Both Japanese and Chinese populations of *Dryopteris chinensis* were recorded to be apogamous triploids (Mitui 1975; Weng 1989a). Besides this cytotype, we found for the first time apogamous tetraploids in Jiangsu Province (Fig. 14, Table 1), which do not apparently differ morphologically from the triploids. One possible relationship of the cytotypes is that apogamous tetraploids originated by crossing between apogamous triploids, which function as male, and sexual diploids, which are not found so far.

Parathelypteris glanduligera — This species is cytologically extremely variable and has been reported to be n=c. 72 (Mitui 1968), 34 (Tsai and Shieh 1983), 36 (Tsai and Shieh 1985), 54 (Weng 1985a), 68 (Weng and Qiu 1988) and 2n=108 (Matsumoto and Nakaike 1990), indicating the basic chromosome number x=27, 34, and 36. Our observation showed obviously that it is x=40 for Chinese populations (Fig. 27). This is also the case with the basic numbers of Thelypteridaceae, for which the numbers x=27, (28), 29, 30, 31, 32, 34, 35, and 36 are found (Lovis 1977).

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Endnote

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中藤成実，加藤雅啓，劉　保東: **江蘇，浙江省（中国）産シダ類の細胞分類学的研究**

これまで中国のシダ類の染色体数と生殖様式について多くの研究がなされており，加藤ら（Kato et al. 1992）も雲南省の90種について報告した．本稿は1991年，1992年に中国東部の江蘇，浙江省から収集した材料を用いて31種2変種のシダ類の染色体数と生殖様式を調べた結果を報告する．その中には種，変種あるいはサイトタイプについて新知見を含む．アマクサシダの2倍体は琉球，台湾，中国東部の限られた地域に分布するが，4倍体は日本，朝鮮南部，中国南部，東部に広く分布する．フモトシダの基本染色体数はx＝42であり，クジャクフモトシダは3倍体雑種である．ヤブソテツには無配生殖3倍体の他に有性生殖2倍体があることがわかった．オオイタチシダの中国東部の集団は，日本の集団と同じく3倍体の他に2倍体無配生殖個体を含み，ミサキカグマは3倍体以外に4倍体の無配生殖個体があることがわかった．ハシゴシダの基本数はこれまでx＝27，34，36が報告されていたが，今回の観察ではx＝40であった．